Bangladesh J. Bot. **47**(4): 1001-1005, 2018 (December) **- Short communication**

DETECTION OF MYCOFLORA AND MYCOTOXIN IN RAW PEANUT *ARACHIS HYPOGEA* L. KERNELS IN BANGLADESH

MD MANIRUZZMAN KHANDAKER*, TASMILUR RAHMAN, MD MATIUR RAHIM[1] AND MD TARIQUL HASSAN[1]

Department of Botany, Jagannath University, Dhaka-1100, Bangladesh

Keywords: Peanut, Mycoflora, Mycotoxin.

Abstract

Raw peanut kernel samples were collected from 13 areas of Bangladesh for determination of mycoflora and mycotoxin. Fungi associated with the tested samples throughout the investigation were *Aspergillus flavus*, *A. niger*, *Aspergillus* sp. (1), *Aspergillus* sp. (2), *Penicillium* sp., *Fusarium* sp., *Rhizopus* sp. and *Curvularia* sp. *A. flavus* was the most common fungus followed by *A. niger*. Colonies of fungi were found to form 79.81 to 98.10% of raw peanut kernels. Out of the 13 samples, eight were found to be contaminated with 11.91 to 182.6 ppb of total aflatoxins and five samples were free from aflatoxins.

The peanut (*Arachis hypogaea* L.) belonging legume family, Fabaceae is one of the major oilseed crops of the world. Peanuts can be consumed as raw, roasted or mixed with other foods or in different processed forms. Recently, peanuts have gained much attention as functional food because it has high protein and energy values and it is suitable for producing other food products. Peanuts contain all the essential amino acids necessary for normal body growth and metabolism. A major challenge in peanut production is fungal contamination. Mycoflora means the fungi characteristic of a particular habitat or environment. Mycotoxins are secondary metabolites produced by microscopic filamentous fungi, which can develop on food crops (Milicevic *et al*. 2010). Mycotoxin attracts worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade (Wagacha and Muthomi 2008). Mycotoxins are mainly produced by fungal species belonging to the genera *Aspergillus*, *Penicillium* and *Fusarium* which are ubiquitous in the environment (Klich 2007). The type and level of mycotoxin production result from the interactions of fungi, host and the environment (Pitt 2000). It has been estimated that 25% of crops produced worldwide are contaminated each year with unacceptable levels of mycotoxins during food production, processing, transport and storage (Kamika and Takoy 2011). Aflatoxins are a group of mycotoxins which are chemically similar to toxic fungal metabolites produced by certain moulds of the genus *Aspergillus* growing on a number of raw food commodities. Aflatoxins are now known to be mainly produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius* (Do and Choi 2007). There are about 20 known aflatoxins but only four of them (aflatoxins B1, B2, G1 and G2) are widely studied because of their toxic effects. Aflatoxin B1 is the most pernicious of these toxins (Wangikar *et al*. 2005). Aflatoxins are more prevalent in tropical and sub-tropical areas where environmental conditions, namely high temperature and humidity prevail, which favour the growth of fungi and production of mycotoxins on the crops. Peanut seeds are good substrate for growth and subsequent aflatoxin production by aflatoxigenic fungi (Xue *et al*. 2003). In Bangladesh peanut is very popular and is consumed by all walks of people. But its mycolfora and mycotoxin levels are still unknown. So the present experiment was conducted to find out the mycoflora association of peanuts and determination of mycotoxin level in raw peanut kernels which are used in many forms of peanut products.

*Author for correspondence: <maniruzzamanbot@gmail.com>. [1]IFST, BCSIR, Dhaka, Bangladesh.

Raw peanut were collected from 13 different locations i.e. Thataribazar, Mirpur, Rampura, Rangpur, Jamalpur, Santinagar, Munshigonj, Faridpur, Joypurhat, Dinajpur, Tangail, Gazipur and Narayangonj during January to May, 2016 for the detection of peanut born fungi and its mycotoxins. The collected samples were thoroughly mixed and divided into two units where one unit of 300 g from each sample was tested for mycoflora using two International Seed Testing Authority (ISTA) recognized media, PDA and blotter. Another unit was tested for mycotoxin detection. Each of 300 g samples for detection of mycoflora were divided into two parts. One part was tested in blotter and another part was in PDA media. For the detection of mycoflora the raw peanut kernels were surface sterilized by sodium hypochloride (NaOCl) and rinsed thrice in sterile distilled water and then air dried. The air dried peanut kernels were kept in three layered sterilized blotting paper in Petri dishes (90 mm dia.) soaked with sterilized water at the rate of 6 seeds per plate. The plates were incubated at room temperature (20 - 23^0C) for four days. The incubated plates were examined for determination of mycoflora and their frequencies. Same number of kernels were plated in PDA. Mycotoxin level of peanut kernels samples were analyzed by standard HPLC (High Performance Liquid Chromatography, Model No. Agilent: 1100 series) method of Food Toxicology Research Section of IFST BCSIR, Dhaka which is validated according to the EC decision 2002/657/EC.

HPLC condition

a. Mobile phase = Acetonitrile: Methanol: Water = 22.5: 22.5: 55

b. Column: C-18,25 cm × 4.6 mm (10 µm packing).

c. Flow rate: 1.5 ml/min.

d. Column temperature: 30°C.

e. Injection volume: 20 µl.

f. Excitation wavelength: 365 nm.

g. Emission wavelength: 418 nm.

h. Software: Agilent Chem Station for 3D systems. Rev. A. 02.

Table 1. Per cent of fungal colonies formed by different genera in 13 peanut samples.

Sample No.	Area	% of colony formed by fungal genera								Total
		A.F.	A.N.	A1	A2	Pen	Fu	Rz	Cur	
1	Thataribazar	50.05	24.43	15.09	-	5.76	3.58	1.09	-	100
2	Mirpur	43.32	37.82	-	10.36	3.94	2.28	1.97	0.31	100
3	Rampura	35.16	37.76	-	22.58	1	2.8	0.7	-	100
4	Rangpur	44.67	38.3	9.18	-	2.79	4.52	0.27	0.27	100
5	Jamalpur	30.96	38.77	26.7	-	-	1.38	2.19	-	100
6	Santinagar	32.51	19.20	41.97	-	0.83	-	5.08	0.41	100
7	Munshiganj	35.35	48.26	9.65	-	0.81	3.14	2.21	0.58	100
8	Faridpur	39.24	57.14	-	-	1.72	0.86	1.04	-	100
9	Joypurhat	51.20	40.29	-	-	-	2.41	6.1	-	100
10	Dinajpur	43.97	32.76	-	-	1.72	14.65	6.9	-	100
11	Tangail	43.36	35.92	6.16	-	0.96	4.04	9.03	0.53	100
12	Gazipur	54.57	35.35	-	-	-	6.32	3.76	-	100
13	Narayangonj	49.44	40.58	-	-	0.4	6.55	3.03	-	100
Total (%)		43.56	37.25	7.53	2.73	1.45	3.96	3.37	0.15	100

A.F. = *Aspergillus flavus,* A.N. = *Aspergillus niger,* A1 = *Aspergillus* sp. (1), A2 = *Aspergillus* sp. (2), Pen = *Penicillium* sp., Fu = *Fusarium* sp., Rz = *Rhizopus* sp. and Cur = *Curvularia* sp.

Clolonies of *Aspergillus flavus*, *A. niger* and *Rhizopus* sp. were commonly observed in all the 13 samples followed by *Fuzarium* sp. in 12 samples, *Penicillium* sp. in 10 samples, *Aspergillus* sp. 1, and *Curvularia* in 6 samples and *Aspergillus* sp. (2) in 3 samples. *A. flavus* was most occurred fungal species which was 43.56% of the total colonies followed by *A. niger* (37.25%). *Curvularia* sp. was the least occurred fungal flora with 0.15% (Table 1).

Fungal colonies formed in PDA ranged from 47.67 to 56.90% while it was 43.10 to 52.33% in blotters. Most of raw kernels were infected and fungal colonies were formed from 79.81 to 98.1% of the kernels and only 1.99 to 20.19% kernels were uninfected. Sample 3 was most infected and sample 9 was least infected kernels (Table 2).

Table 2. Per cent of fungal colony formation in kernels of 13 peanut samples on PDA and blotter media and per cent of kernel infection.

Sample No.	Area	% colony formation		% infected/uninfected kernels	
		on PDA	on Blotter	Infected	Uninfected
1	Thatari bazar	55.81	44.19	96.27	3.73
2	Mirpur	51.30	48.70	97.3	2.7
3	Rampura	51.45	48.55	98.1	1.99
4	Rangpur	54.03	45.97	90.23	9.77
5	Jamalpur	52.70	47.30	89.72	10.28
6	Santinagar	54.87	45.13	90	10
7	Munshiganj	47.67	52.33	91.76	8.24
8	Faridpur	50.00	50.00	79.81	20.19
9	Joypurhat	49.28	50.72	95.32	4.68
10	Dinajpur	56.90	43.10	85.5	14.5
11	Tangail	48.78	51.22	93.64	6.36
12	Gazipur	48.68	51.32	95.15	4.85
13	Narayangonj	49.92	50.08	97.78	2.22

All of the 13 peanut samples were tested for aflatoxin detection. Various amounts of aflatoxin were detected from eight samples *viz.* 1, 2, 3, 6, 7, 8, 11 and 13. The highest 182.62 ppb of total aflatoxins were detected in sample 1 which includes aflatoxin B1 = 5.82 ppb, B2 = 0.15 ppb, G1 = 176.16 ppb and G2 = 0.49 ppb. The second highest total aflatoxin 62.91 ppb were detected in sample 13 where, aflatoxin B1 = 5.32 ppb, B2 = 0.08 ppb, G1 = 57.19 ppb and G2 = 0.32 ppb, it was followed by sample 3, where 62.89 ppb of total aflatoxins were detected, which were included aflatoxin B1 = 4.91 ppb, G1 = 57.70 ppb, aflatoxin G2 = 0.28 ppb, and aflatoxin B2 was not detected in this sample. All the eight aflatoxins detected peanut samples exceeded the European Union permissible limit for total aflatoxin. European Union permissible limit for total aflatoxinis is four ppb (Commission Regulation No. 165/2010). Aflatoxins were not detected from the remaining five samples *viz.* sample 4, 5, 9, 10 and 12 (Table 3).

Mycoflora association was detected in peanut seed samples from five different Governorates in Egypt, namely Aswan, Giza, Behera, Monofya and Sharkia. The fungi isolated from the samples belong to four fungal genera i.e. *Aspergillus, Fusarium, Penicillium* and *Rhizopus.* Agar plate (PDA) medium was an enhanced method for seed health testing than the blotter test method

Table 3. Aflatoxin analysis result of thirteen raw peanut samples.

Sample no.	Collection area	Aflatoxins (ppb)				Total amount of aflatoxins (ppb)
		B1	B2	G1	G2	
1	Thataribazar	5.82	0.15	176.16	0.49	182.62
2	Mirpur	3.73	-	6.51	1.67	11.91
3	Rampura	4.91	-	57.70	0.28	62.89
4	Rangpur	-	-	-	-	-
5	Jamalpur	-	-	-	-	-
6	Santinagar	3.64	-	9.31	-	12.95
7	Munshiganj	4.26	-	12.05	-	16.31
8	Faridpur	12.03	2.87	7.35	-	22.25
9	Joypurhat	-	-	-	-	-
10	Dinajpur	-	-	-	-	-
11	Tangail	1.89	-	40.31	-	42.20
12	Gazipur	-	-	-	-	-
13	Narayangonj	5.32	0.08	57.19	0.32	62.91

and gave higher numbers of fungal colony (Embaby and Mona 2006, Embaby *et al.* 2008). Similar results and techniques were observed from this investigation of raw peanut samples. Oliveira *et al*. (2009) observed in their experiment that 44.2% of 240 peanut samples analyzed were positive for aflatoxin at levels between 0.5 and 103.8 µg/kg. In the present study, out of 13 raw peanut samples eight samples (61.54%) were contaminated with various amounts (11.91 - 182.62 ppb) of aflatoxins, which corroborate the above finding.

Acknowledgements

The authors acknowledge the financial support of Ministry of Science and Technology, Govt. of People's Republic of Bangladesh to carry out the research.

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(Manuscript received on 24 February, 2018; revised on 13 July, 2018)